

WE CLAIM:

1. A method of detecting the activity of an enzyme that operates on an enzyme substrate to form an enzyme product in a sample, comprising:

5 contacting the substrate with a binding partner that specifically binds to the substrate or to the product but not to both, where the binding partner includes a metal ion that is required for binding between the binding partner and the substrate or product;

contacting the substrate with the enzyme;

10 detecting a response indicative of the extent of binding between the substrate or the product and the binding partner without separating the bound substrate or product from the unbound substrate or product; and

correlating the response with the activity of the enzyme.

15 2. The method of claim 1, where the metal ion is a tricationic metal ion.

3. The method of claim 2, where the tricationic metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

4 The method of claim 3, where the tricationic metal ion is Ga^{3+} .

20 5. The method of claim 2, where the binding partner further includes a dicationic metal ion.

6. The method of claim 1, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the
sample; and

measuring a detectable luminescence response, where the detectable luminescence
5 response is indicative of the extent of binding between the substrate or product and the
binding partner.

7. The method of claim 6, where at least one of the substrate, product, and
binding partner is luminescent.

8. The method of claim 6, where the condition is light capable of inducing
photoluminescence.

9. The method of claim 8, where the detectable luminescence response is
luminescence intensity.

10. The method of claim 8, where the detectable luminescence response is
luminescence polarization.

11. The method of claim 8, where the detectable luminescence response is
luminescence resonance energy transfer.

12. The method of claim 6, where the condition is electrochemical energy capable of inducing electrochemiluminescence.

13. The method of claim 1, where the substrate is a polypeptide, and where the
5 substrate and product are related by a posttranslational modification.

14. The method of claim 13, where the posttranslational modification is phosphorylation or dephosphorylation of the polypeptide.

15. The method of claim 1, where the substrate is a nucleotide, and where the
10 substrate and product are related by a cyclization or decyclization of the nucleotide.

16. The method of claim 1, where the binding partner further includes a
15 macromolecule.

17. The method of claim 1, where the binding partner further includes a
nanoparticle.

18. The method of claim 1, where the binding partner further includes a
20 quencher or an energy transfer partner.

19. The method of claim 1, the sample being supported by a sample holder, where the binding partner is linked to the sample holder.

20. The method of claim 1, where the enzyme is selected from the group consisting of kinases and phosphatases.

21. The method of claim 1, where the enzyme is selected from the group consisting of cyclases and phosphodiesterases.

22. The method of claim 1, where the substrate includes a phosphorylated polypeptide or a nonphosphorylated polypeptide.

23. The method of claim 1, where the substrate includes a cyclized nucleotide or a noncyclized nucleotide.

24. The method of claim 1, further comprising:
contacting the substrate and enzyme with a candidate compound; and
determining the ability of the candidate compound to enhance or inhibit enzyme activity by its effects on the response.

25. The method of claim 1, the specific binding being characterized by a binding coefficient, where the binding coefficient is no larger than about 10^{-8} M.

26. The method of claim 1, further comprising:

providing a sample holder having a plurality of sample sites supporting a corresponding plurality of samples; and

repeating the steps of contacting, detecting, and correlating for the plurality of

5 samples.

27. A method of detecting phosphorylation or nonphosphorylation of a polypeptide in a sample, comprising:

contacting the polypeptide with a binding partner that specifically binds to the phosphorylated polypeptide or to the nonphosphorylated polypeptide but not to both, where the binding partner includes a metal ion that is required for binding between the binding partner and the phosphorylated polypeptide or nonphosphorylated polypeptide;

detecting a response indicative of the extent of binding between the polypeptide and the binding partner without separating the bound polypeptide from the unbound polypeptide; and

correlating the response with the extent of phosphorylation or nonphosphorylation of the polypeptide, or with the activity of an enzyme that affects phosphorylation or nonphosphorylation of the polypeptide.

28. The method of claim 27, where the metal ion is a tricationic metal ion.

29. The method of claim 28, where the tricationic metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

30. The method of claim 29, where the tricationic metal ion is Ga^{3+} .

31. The method of claim 28, where the binding partner further includes a dicationic metal ion.

32. The method of claim 27, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the sample; and

measuring a detectable luminescence response, where the detectable luminescence response is indicative of the extent of binding between the polypeptide polypeptide and the binding partner.

33. The method of claim 32, where at least one of the phosphorylated polypeptide, nonphosphorylated polypeptide, and the polypeptide is luminescent.

34. The method of claim 32, where the condition is light capable of inducing photoluminescence.

35. The method of claim 34, where the detectable luminescence response is luminescence intensity.

5 36. The method of claim 34, where the detectable luminescence response is luminescence polarization.

37. The method of claim 34, where the detectable luminescence response is luminescence resonance energy transfer.

38. The method of claim 32, where the condition is electrochemical energy capable of inducing electrochemiluminescence.

39. The method of claim 27, where the polypeptide includes fewer than about 50 amino acids.

40. The method of claim 27, where the binding partner further includes a macromolecule.

20 41. The method of claim 27, where the binding partner further includes a nanoparticle.

42. The method of claim 27, where the binding partner further includes a quencher or an energy transfer partner.

43. The method of claim 27, the sample being supported by a sample holder,
5 where the binding partner is linked to the sample holder.

44. The method of claim 27, where the binding partner binds to the phosphorylated polypeptide.

45. The method of claim 27, where the binding partner is not an polypeptide.

46. The method of claim 27, where the enzyme catalyzes addition or cleavage
of a phosphate group to or from a protein, further comprising contacting the polypeptide
with the enzyme prior to the steps of contacting, measuring, and correlating.

47. The method of claim 46, where the enzyme is a kinase.

48. The method of claim 46, where the enzyme is a phosphatase.

115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

49. The method of claim 46, further comprising:
contacting the polypeptide and enzyme with a candidate compound; and
determining the ability of the candidate compound to enhance or inhibit
phosphorylation or dephosphorylation of the polypeptide by its effects on the response.

5

50. The method of claim 27, the specific binding being characterized by a
binding coefficient, where the binding coefficient is no larger than about 10^{-8} M.

51. The method of claim 27, further comprising:
providing a sample holder having a plurality of sample sites supporting a
corresponding plurality of samples; and
repeating the steps of contacting, detecting, and correlating for the plurality of
samples.

004455-043701
T92270" 5594460

52. A method of detecting phosphorylation or nonphosphorylation of a substrate in a sample, comprising:

contacting the substrate with a binding partner that specifically binds to the phosphorylated substrate but not to the nonphosphorylated substrate, where the binding partner is not a polypeptide;

detecting a response indicative of the extent of binding between the substrate and the binding partner without separating the bound substrate from the unbound substrate; and

correlating the response with the extent of phosphorylation or nonphosphorylation of the substrate, or with the activity of an enzyme that affects phosphorylation or nonphosphorylation of the substrate.

53. The method of claim 52, where the binding partner includes a metal ion that is required for binding between the binding partner and the substrate.

54. A method of detecting phosphorylation or nonphosphorylation of a substrate in a plurality of samples, comprising:

providing a sample holder having a plurality of discrete sample sites for supporting a corresponding plurality of samples, where each sample site includes at least one assay surface adapted to bind specifically to the phosphorylated substrate but not to the nonphosphorylated substrate;

contacting the assay surface in at least one assay site with a sample having the substrate;

detecting a response indicative of the extent of binding between the substrate and the assay surface; and

correlating the extent of binding with the extent of phosphorylation or nonphosphorylation of the substrate, or with the activity of an enzyme that affects phosphorylation or nonphosphorylation of the substrate.

55. The method of claim 54, where the sample holder is a microplate.

56. The method of claim 54, where the assay surface includes a metal ion that is required for the binding between the substrate and the assay surface.

57. The method of claim 54, where the metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

58. The method of claim 54, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the
sample; and

measuring a detectable luminescence response, where the detectable luminescence
5 response is indicative of the extent of binding between the substrate and the binding
partner.

59. The method of claim 58, where the response is luminescence intensity.

60. The method of claim 54, where the response is absorption.

61. The method of claim 54, where the step of detecting a response is
performed without separating the bound substrate from the unbound substrate.

62. The method of claim 61, the sample holder having a bottom surface that
transmits light, where the step of detecting is performed through the bottom surface.

63. The method of claim 61, further comprising adding a blocking reagent to
reduce background prior to the step of detecting a response.

00444555-0427015

64. The method of claim 54, further comprising washing the sample to remove any substrate nucleotide not bound to the assay surface prior to the step of detecting a response.

5 65. The method of claim 54, further comprising eluting the bound substrate from the assay surface prior to the step of detecting a response.

66. The method of claim 54, where the enzyme is a kinase or a phosphatase.

67. A method of detecting cyclization or noncyclization of a nucleotide in a sample, comprising:

contacting a nonradioactive nucleotide with a binding partner that specifically binds to a cyclized nucleotide or to a noncyclized nucleotide but not to both, substantially without regard to the nucleoside portion of the nucleotide;

detecting a response indicative of the extent of binding between the nucleotide and the binding partner; and

correlating the response with the extent of cyclization or noncyclization of the nucleotide, or with the activity of an enzyme that affects cyclization or noncyclization of the nucleotide.

094455 "04205
094455 "04205

68. The method of claim 67, where the binding partner includes a metal ion that is required for binding between the binding partner and the cyclized nucleotide or noncyclized nucleotide.

5 69. The method of claim 68, where the tricationic metal ion is a tricationic metal ion.

70. The method of claim 69, where the tricationic metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

71. The method of claim 69, where the binding partner further includes a dicationic metal ion.

72. The method of claim 67, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the sample; and

measuring a detectable luminescence response, where the detectable luminescence response is indicative of the extent of binding between the nucleotide and the binding partner.

20 73. The method of claim 72, where at least one of the cyclized nucleotide, noncyclized nucleotide, and the nucleotide is luminescent.

74. The method of claim 72, where the condition is light capable of inducing photoluminescence.

5 75. The method of claim 74, where the detectable luminescence response is luminescence intensity.

76. The method of claim 74, where the detectable luminescence response is luminescence polarization.

77. The method of claim 74, where the detectable luminescence response is luminescence resonance energy transfer.

78. The method of claim 72, where the condition is electrochemical energy capable of inducing electrochemiluminescence.

79. The method of claim 67, where the nucleotide includes an adenine or a guanine.

20 80. The method of claim 67, where the binding partner further includes a macromolecule.

81. The method of claim 67, where the binding partner further includes a nanoparticle.

82. The method of claim 67, where the binding partner further includes a
5 quencher or an energy transfer partner.

83. The method of claim 67, the sample being supported by a sample holder, where the binding partner is linked to the sample holder.

84. The method of claim 67, where the binding partner binds to the cyclized nucleotide.

85. The method of claim 67, where the binding partner is not an polypeptide.

86. The method of claim 67, where the enzyme catalyzes cyclization or decyclization of a nucleotide, further comprising contacting the nucleotide with the enzyme.

87. The method of claim 86, where the enzyme is a phosphodiesterase.

88. The method of claim 86, where the enzyme is a cyclase.

89. The method of claim 86, further comprising:
contacting the nucleotide and enzyme with a candidate compound; and
determining the ability of the candidate compound to enhance or inhibit
cyclization or decyclization of the nucleotide by its effects on the response.

5

90. The method of claim 67, where the step of detecting a response is
performed without separating the bound nucleotide from the unbound nucleotide.

91. The method of claim 67, further comprising washing the sample to remove
any nucleotide not bound to the binding partner prior to the step of measuring the
detectable luminescence response.

92. The method of claim 67, the specific binding being characterized by a
binding coefficient, where the binding coefficient is no larger than about 10^{-8} M.

93. The method of claim 67, further comprising:
providing a sample holder having a plurality of sample sites supporting a
corresponding plurality of samples; and
repeating the steps of contacting, detecting, and correlating for the plurality of
samples.

20

Pat
C1